The Action of X-Rays on the Developing Chick.

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(Communicated by W. H. Gaskell, M.D., F.R.S. Received November 11, 1910,—Read January 19, 1911.)

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This research was undertaken at the suggestion of Prof. Grünbaum, in order to discover whether any changes were produced in the chick embryo under the action of X-rays. Embryos of various ages were examined, both directly after exposure to doses of various amounts, and also at later periods. In none were any observations obtained of changes either in the embryo as a whole or in any particular organ, with the exception of one which affected all the tissues so exposed.

It was found that X-rays had a general effect on the rate of division of the cells of the embryo, causing a diminution in the number of mitoses. This diminution was very evident in embryos exposed to long doses; for instance, chicks incubated for three days and then given a two hours' exposure, showed so marked a diminution that a considerable search was required to find any mitotic figures, while in an embryo of three days, exposed for four hours, no mitotic figures could be found.

In order to discover whether this diminution was progressive from the beginning of the exposure, or whether initially an increase of mitotic activity occurred, small doses of 5 and 10 minutes were given to embryos of four days. These were used with their controls for the purpose of making quantitative counts. The fore-brain was selected as the most suitable tissue for counting owing to the regular arrangement of the cells. That portion only was counted which was of fairly uniform thickness, and, as far as possible, median sagittal sections only were used for counting, in order to count only cells cut in their principal plane. The sections were cut 5μ thick and cells were counted by their nuclei. The results of the counts are shown in Table I. The eggs were exposed at about 20 cm. distance from the anti-cathode.

The figures obtained do not show very wide variations in each individual count, so that they should be sufficiently reliable to establish the fact that there is a progressive diminution of mitotic activity even with small doses. Counts were also attempted on the alimentary canal and the liver, but the difficulties of counting and the number of cells available did not give reliable figures; the same tendency to diminution was, however, observed.

Further evidence of a different kind supporting the view that the diminution occurs equally in all tissues will be given later in this paper.

Table I.

Expt. No.	Duration of X-ray dose.	Number of cells counted.	Mitoses observed.	Mitotic index or number per 1000.
1	Control	2,738	79	28·8
2	,,	2,548	75	29·5
3	,,	2,576	77	29·9
4	,,	2,412	72	29·8
Total		10,274	303	29·5
1	5 minutes	3,063	72	23·5
2	,,	2,592	62	23·9
3	,,	2,625	62	23·6
Total		8,280	196	23·7
1	10 minutes	3,769	66	17·5
2	,,	3,953	75	18·8
3	,,	1,877	39	20·7
Total		9,599	180	18·7

Other specimens were incubated for three days and then X-rayed for 15 minutes, 30 minutes, and one hour respectively. The progressive diminution in mitotic activity was evident in these, though no actual counts were made. It appears, therefore, that a progressive diminution of mitotic activity takes place, under the influence of X-rays, in a rapidly growing organism such as the chick embryo.

These results are contrary to those arrived at by Cattley* in the growing root tips of plants. He found that an increasing stimulatory effect was obtained with increasing lengths of exposure up to one of 30 minutes' duration. From this point there was diminution, but not to a figure below that obtained in the controls until the exposure had lasted for two hours. His exposures were at a somewhat greater distance, 25 cm. from the anti-cathode, but this would not be a factor of sufficient importance to account in any way for the different type of results obtained.

It is possible that an initial stimulant effect may occur in the chick embryo also; it must, however, be very brief; smaller doses than one of five

* 'Journ. of Path. and Bact.,' April, 1909, vol. 13, p. 380.

minutes' duration, in order to settle this point, have not been given in these experiments.

The effect of this diminution of mitotic activity was then tried on the development of the embryo. Daily exposures of varying length were given to eggs which were incubated for the full 21 days. These were opened on the twenty-third day if hatching had not taken place. The results of various experiments are grouped together in Table II. In all these experiments the controls hatched out at the proper time, with the exception of three, which were apparently sterile.

Table II.

Length of daily exposure.	Distance from anti-cathode.	Results.	Average growth in days.
2 hours	15 cm.	6 eggs with no growth	0
30 minutes	12 ,,	2, 3rd day; 3, no growth	1·25
30 ,,	20 ,,	3, 5th day; 1, 3rd; 1, no growth	4·5
20 ,,	20 ,,	1, 10th day; 1, 7th; 1, 6th; 1, 5th; 1, 3rd	6·2
15 ,,	12 ,,	1, 14th day; 1, 13th; 1, 9th; 1 sterile	12
10 ,,	12 ,,	2 hatched; 1, 19th; 1, 17th	19·5
10 ,,	20 ,,	2 hatched; 1 opened on 17th day; 2 sterile	21
5 ,,	12 ,,	3 hatched; 1, 8th day	21

In the calculations the sterile eggs and the one in the last series which only reached the eighth day's development are neglected, this latter being decomposed.

The variations in the individual groups may be partly due to the fact that each group of eggs was exposed together in a box; the eggs were not, therefore, all equidistant from the anti-cathode. Also the X-ray tube did not give a constant dose throughout the experiments. Attempts were made to measure the dose by means of Sabaraud's pastilles, but the results were unsatisfactory, especially in the case of the short exposures.

The table shows that a daily exposure of a certain length stops further development of the embryo at a certain stage. The stage of development bears an inverse ratio to the exposure given. The critical dose, which renders the hatching of the chick doubtful, is a daily exposure of 10 minutes at 12 cm. distance in this series of experiments. Those exposed to a smaller daily dose all successfully hatched out. Those given a daily five minutes' exposure hatched out a few hours earlier than their controls but this was probably due to their shorter stay outside the incubator and their consequent shorter period for cooling. The controls were always left out 20 minutes at least, while the others only stayed out while being

exposed. This is not, therefore, taken as any evidence of any stimulating effect on their growth.

The question then arose as to whether the effect was cumulative or recovery was complete. A further experiment was undertaken, and the treatment was stopped on the twelfth day. The results are shown in Table III.

Table III.

Length of daily exposure.	Distance from anticathode.	Results.	Average growth in days.
20 minutes	15 cm.	1, 5th; 1, 7th; 1, 9th; 1, 11th; 1, 13th	9
15 „	15 „	2 hatched; 1, 19th; 1, 4th; 2 sterile	20
10 „	15 „	3 hatched; 3 sterile	21
5 „	15 „	6 hatched	21

In this experiment, therefore, 20 minutes' exposure killed on or before the twelfth day, that is to say, death occurred while exposure was still going on. The average development of nine days agrees fairly well with Table II, in which a 20 minutes' dose gave development to six days. The fact that a different X-ray tube was being used may also, to some extent, account for the difference.

Those given a 15 minutes' exposure were able to hatch out, though in the previous experiments this did not occur. These embryos had not yet reached the critical stage of development at which an X-ray dose of 15 minutes takes effect; they therefore survived, and were able to hatch out at the usual time. The embryo which only reached the fourth day of development is neglected, as this was probably accidental.

Those exposed for 10 minutes and 5 minutes hatched out as in the previous experiments at the usual 21 days. The evidence from this experiment therefore tends to show that the effect is not a cumulative one, but that there is a critical point at which a certain exposure stops development.

Further experiments were intended to support this view. Eggs were to be incubated up to a certain day and then given a single exposure of length differing in the case of each group. In such a series the particular length of exposure which would just prevent further development ought to agree with that obtained from Table II, if the effect is non-cumulative. All those receiving a smaller exposure should hatch out. It has, however, at present not been possible to continue these experiments, so that this evidence is as yet lacking. Additional facts supporting the non-cumulative view have been obtained from embryos opened at various stages of development while under-

going daily exposures. If alive, they were always at the correct stage of development; none gave evidence of any retardation. The fact that the X-rayed embryos which received small doses hatched at the usual time also implies that the recovery from the X-ray effect is complete for each exposure.

The hatched chicks were kept alive to watch their further development. Unluckily, the first to hatch out under X-ray treatment did so in the late autumn, and the conditions were unfavourable for their proper development; but nevertheless no difference could be observed between them and their controls. They were killed and examined at various stages, but no differences were discovered in the development of any of their organs. This is in accord with the microscopical evidence given earlier in this paper: the X-ray action is uniform on all tissues.

Particular attention was paid to the generative organs, but neither macroscopically nor microscopically did they differ from the controls in any way. The chicks obtained from the experiment in Table III are at present being brought up with the intention of testing their breeding capabilities, to see if any action has occurred on their generative cells. They are at present all normal in appearance.

The meaning of these experiments, and the probable action of X-rays on growing tissues, may now be considered. It has been shown by Minot,* by actually counting the number of mitoses per 1000 cells in the various tissues of embryos of different ages, that throughout embryonic life a rapid diminution of mitotic activity is going on. He calls the figures obtained the mitotic index for that particular tissue.

This mitotic index varies at any particular stage of development according to the rapidity of growth of the particular organ at that moment, but this variation is not sufficient to obscure the general diminution of the index which is taking place. The effect of X-rays is to bring about rapidly a progressive lowering of this mitotic index in all the tissues of the developing embryo. This lowering is not permanent, and is completely recovered from so long as it has not been of too great a degree. If, however, the exposure has been of a certain critical length, recovery no longer takes place, and the embryo dies. This critical dose depends upon the stage of development reached by the embryo, that is to say, upon the value of the general mitotic index at that stage; the larger the mitotic index is—that is to say, the greater the reproductive activity—the larger is the X-ray dose required to prevent further development.

The X-rays are, therefore, directly antagonistic to the reproductive activity

* "The Problem of Age, Growth, and Death," 'Popular Science Monthly,' 1907, vol. 71, p. 510.

of the cell, and act in such a way as to lower, and finally to destroy, its power of reproduction.

I am greatly indebted to Prof. Grünbaum for the suggestion of this research, for the use of the apparatus, and for his advice and encouragement. For some months previously he had been working at this subject, and had been assisted by a grant from the Grant Committee of the Royal Society. I am fully aware that this paper is of the nature of a preliminary communication, and that the investigation is by no means complete. As, however, I am unlikely to be able to continue it at present, I have thought it best (with the concurrence of Prof. Grünbaum) to publish it in its present unfinished condition rather than to wait for a further opportunity to complete the investigation.

Summary.

1. The action of X-rays is to lower the mitotic activity of growing tissues.

2. If this diminution is not too great, complete recovery occurs.

3. If the diminution is above a certain degree, recovery does not take place.

4. The critical dose, which just prevents recovery, varies with the stage of development of the embryo, decreasing as the mitotic index decreases.

5. No other macroscopic or microscopic changes have been observed to take place under the action of X-rays, either in the proportions or structure of the various tissues.